**Master 1 or Master 2 internship project**

**Year 2024-2025**

**Laboratory/Institute:** Institut de Biologie Structurale **Director:** Winfried Weissenhorn

**Team:** Viral Replication Machines **Head of the team:** Marc JAMIN

**Name and status of the scientist in charge of the project:** Pr Marc JAMIN

 **HDR: yes X no ☐**

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**Program of the Master’s degree in Biology:**

X Microbiology, Infectious Diseases and Immunology **X** Structural Biology of Pathogens

**☐** Physiology, Epigenetics, Differentiation, Cancer **☐** Neurosciences and Neurobiology

**Title of the project: Rabies virus nucleocapsid assembly**

Objectives (up to 3 lines):

Purify the unassembled nucleoprotein-phosphoprotein complex (N0-P) of rabies virus and characterize the assembly process in the absence and presence of different RNAs. This will be done (1) by monitoring the kinetics using different probes (light scattering, fluorescence) and (2) by establishing equilibrium binding curves.

Abstract (up to 10 lines):

The RNA genome of all nonsegmented negative-sense RNA viruses, including rabies virus, is encapsidated by the nucleoprotein forming a long, helical nucleocapsid. The assembly of the nucleocapsid is an essential step in the replication of all these viruses and it requires the production of unassembled viral nucleoprotein in complex with its chaperone, the phosphoprotein (N0-P complex). Upon genome replication, the nucleoprotein is tranfered from the P protein to the nascent RNA and it assembles into a long homopolymer. We aims at reconstituting this process in vitro to understand in molecular details the different steps involved (release of P, assembly of N, conformational change of N,…). We have devised a strategy to produce a functional N0-P complex and have shown that by mixing it with RNA, we can form nucleocapsid. The project is to measure kinetics and equilibrium binding curves and test the effects of different parameters such as temperature, salt concentration and the length and sequence of RNA by using various probes

Methods (up to 3 lines):

Expression (insect cells) and purification of proteins. Optical spectroscopies (absorbance, fluorescence, circular dichroism), light scattering, size-exclusion chromatography combined with static light scattering (SEC-MALLS).

Up to 3 relevant publications of the team:

(1) Gérard et al. (2022) ‘Structure and Dynamics of the Unassembled Nucleoprotein of Rabies Virus in Complex with Its Phosphoprotein Chaperone Module’, *Viruses*, 14(12). doi:[10.3390/v14122813](https://doi.org/10.3390/v14122813).

(2) Bourhis et al. (2022) ‘Structural Dynamics of the C-terminal X Domain of Nipah and Hendra Viruses Controls the Attachment to the C-terminal Tail of the Nucleocapsid Protein’, *J Mol Biol*, 434(10). doi:[10.1016/j.jmb.2022.167551](https://doi.org/10.1016/j.jmb.2022.167551).

(3) Yabukarski et al. (2014) ‘Structure of Nipah virus unassembled nucleoprotein in complex with its viral chaperone.’, *Nat Struct Mol Biol*, 21(9). doi:[10.1038/nsmb.2868](https://doi.org/10.1038/nsmb.2868).

Requested domains of expertise (up to 5 keywords):

Protein biochemistry, spectroscopy